Mechanisms of Oral Staphylococcal Enterotoxin B-Induced Emesis in the Monkey (38553)

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Introduction. Staphylococcal enterotoxin B (SEB), isolated from cultures of *Staphylococcus aureus*, produces symptoms of food poisoning in man and other primates (1–3). Purified SEB (>99%) prepared by the method of Schantz *et al.* (4) induces vomiting and diarrhea in monkeys when given either orally or intravenously (iv) (1, 3). Although considerable effort has been expended on attempts to clarify the pathogenesis whereby oral administration of SEB induces vomiting, the physiological mechanisms still remain obscure.

When crude staphylococcal enterotoxin filtrates were administered iv or intraperitoneally (ip) to cats, Bayliss (5) concluded that emesis was induced through a peripheral action involving the vagal nerves. However, oral administration and intraventricular application of the enterotoxin filtrate failed to induce emesis. A subsequent study by Clark *et al.* (6) with a more purified enterotoxin confirmed the findings of Bayliss (5). Sugiyama and coworkers (7, 8) demonstrated that denervation of abdominal viscera by sympathectomy and abdominal vagotomy suppressed vomiting in monkeys after oral or iv challenge with SEB. They believed that a neural mechanism played a role in SEB-induced vomiting. Although these investigators showed that injection of enterotoxin into the fourth ventricle did not produce vomition, destruction of the area postrema gave complete protection from both oral and iv toxin administration. Thus, the question could be raised as to whether

the vomiting reflex caused by oral SEB resulted from a direct action of absorbed enterotoxin on the vomiting center, indirectly by the action of some secondary humoral mediating substance released into the circulation, or from a neural stimulus to the vomiting center originating within the gastrointestinal tract.

Cross-circulated pairs of rhesus monkeys were chosen as an experimental model to investigate the mechanism for the emetic response to orally administered SEB. During cross-circulation, Evans blue dye was employed to evaluate the exchange and distribution of blood between the pair. Blood pH, P_{CO_2}, P_{O_2}, hematocrit, and plasma osmolality were determined at selected intervals throughout the acute study. SEB hemagglutinating antibody (HA) titers were followed subsequently for a 6-wk period in all monkeys (9).

Method. Healthy rhesus monkeys, *Macaca mulatta*, of both sexes, weighing 3.0–4.5 kg, were used. Members of each pair demonstrated negative SEB hemagglutination titers (9) prior to study and were of approximately equal weight. The animals were tranquilized with 20 mg of ketamine hydrochloride intramuscularly (im), premedicated with atropine (0.04 mg/kg, im), and anesthetized with halothane (0.5–1.5%) vaporized during oxygen inhalation. Unilateral femoral artery and vein catheters were placed in each monkey using Teflon vessel tips (Extracorporeal) to which medical silicon tubing was attached according to the method of Chapple *et al.* (10). Monkeys were heparinized immediately following surgery (200 units iv, 700 units subcutaneously) and restrained in chairs for recovery. Food and water were maintained *ad lib.*

Twenty-four hours after surgery, blood samples were taken for hematocrit determinations, and monkeys were cross-circulated by femoral artery to femoral vein intercon-

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[1] In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences—National Research Council. The facilities are fully accredited by the American Association of Accreditation of Laboratory Animal Care.

nections. Each monkey received 300 units of sodium heparin iv, and remaining food was removed from the chair trays. Hematocrit values were measured 1 and 2 hr after initiating cross-circulation. Base-line control arterial samples were obtained from each monkey for plasma osmolality, and Evans blue, as well as for arterial blood pH, P_{CO_2}, and P_{O_2} determinations. Highly purified SEB, Lot 14–30, which has been used extensively in previous studies at this Institute, was dissolved in physiological saline to a final concentration of 1 mg/ml, and a 1 mg/kg dose was given to one monkey of each of five pairs via nasogastric tube. The other monkey in each pair received an equal volume of saline diluent via nasogastric tube. Evans blue dye (0.4 mg) was injected into the SEB recipient through the femoral venous catheter. Monkeys were observed for vomiting or other clinical signs of illness over a 5-hr period. Heparinized arterial samples were taken hourly from each monkey throughout the experimental period. Evans blue in plasma was measured on a spectrophotometer at 620 nm. Blood pH, P_{CO_2}, and P_{O_2} were determined with a Corning pH blood gas analyzer. Hematocrit values were determined by microcentrifugation and plasma osmolality was measured with an osmometer.

In a sixth pair of monkeys, an iv SEB challenge (5 μg/kg) was administered to one monkey and saline to its pairmate. This pair was cross-circulated for 3 hr postchallenge. In all monkeys, catheters were disconnected at the end of the 5-hr experiment and removed under ketamine anesthesia. HA titers were measured thereafter at 2, 4, and 6 wk (9).

Results. Monkeys that vomited after receiving SEB exhibited signs of uneasiness and abdominal discomfort prior to vomition and appeared listless and relatively unaware of usual environmental distractions following the vomition period. On the other hand, paired controls that did not vomit remained alert and aggressive. Vomiting occurred in all recipients of intragastric SEB (Table I), but only in one of the monkeys receiving saline. This particular monkey in pair 1 (Table I) that vomited at 191 min did so without typical premonitory or postvomiting signs. The mean time for the first episode of vomition was 112 min (range, 55–270 min). The mean frequency of vomiting was 3.4 times per oral SEB-recipient monkey over the 5-hr period of observation. Vomiting occurred in the iv SEB-challenged pair in both the saline control and toxin-challenged monkeys at 50 and 95 min, respectively. Although plasma concentrations of Evans blue decreased as a function of time in all monkeys, values were essentially the same for both paired monkeys at any given time (Table II), indicating that there was a complete mixing of blood between the two.

No significant changes were observed in plasma osmolality, blood pH, P_{CO_2}, P_{O_2}, or hematocrit values during the 5-hr period in any monkey (Fig. 1). Four of five oral SEB-recipient monkeys developed HA titers within 6 wk after toxin challenge, while none of their saline-recipient pairmates developed an antibody response to SEB (Table I). In contrast, both monkeys from the iv-challenged pair developed titers by 6 wk postchallenge. The marked HA response in the SEB recipient of pair 4 at 2 wk was unusual

TABLE 1. EFFECT OF ORAL SEB (1 mg/kg) AND IV SEB (5 μg/kg) ON VOMITION TIME AND ANTIBODY RESPONSE IN CROSS-CIRCULATED MONKEYS.

Pair	Vomition time (min post-SEB)		Maximum hemagglutination titer (wk post-SEB)	
	SEB recipient	NaCl control	SEB recipient	NaCl control
Oral				
1	55, 70, 80, 105	191	1:160 (4)	Neg
2	75, 85, 97, 110	0	Neg	Neg
3	95, 110	0	1:320 (4)	Neg
4	65, 80, 85, 100, 103, 130	0	1:1280 (2)	Neg
5	270	0	1:40 (4)	Neg
iv	95	50, 60, 80	1:80 (4)	1:160 (6)

TABLE II. THE RATIO OF PLASMA EVANS BLUE
CONCENTRATIONS EXPRESSED AS SALINE
CONTROL/SEB RECIPIENT.

Pair	Hour				
	1	2	3	4	5
1	1.00	0.98	1.00	0.96	0.99
2	1.01	1.03	0.96	0.93	0.99
3	0.81	1.01	1.04	1.02	1.03
4	1.14	0.99	1.00	0.99	0.98
5	0.99	1.00	1.00	1.00	—
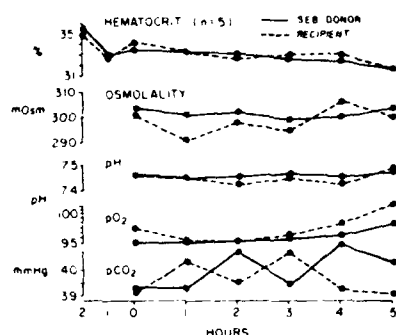
FIG. 1. Effect of oral SEB (1 mg/kg) on hematocrit, plasma osmolality and blood pH, PO_2, and PCO_2 in cross-circulated monkeys.

and suggested an anamnestic response. Further, a positive precipitin reaction at that time also indicated that the monkey, despite its negative titer at the time of study, evidently had previous exposure to SEB antigen and responded with IgG antibody (9).

Discussion. Maintenance of complete and continuous mixing of blood between the two monkeys in each pair was a key problem in the present model. The close agreement between pairmates in their values for hematocrit and for concentration of Evans blue in plasma signified that plasma and blood volumes were evenly distributed between the two monkeys throughout the entire experimental period. Furthermore, the stability of plasma osmolality, blood pH, P_{CO_2}, and P_{O_2} in pairmates indicated that homeostasis was well maintained.

Our findings support the concept that the emetic response to oral SEB occurs entirely as a consequence of a local, gastrointestinally initiated, neural stimulus to the vomiting center. Recipients of very large oral doses of

SEB vomited consistently, while their cross-circulated pairmates generally remained unaffected. Apparently, the massive dose of oral SEB (1 mg/kg) received by one of each pair of monkeys failed to be absorbed in sufficient quantity to induce vomition in the saline pairmate by any possible mechanism.

The absence of an SEB hemagglutinin response in saline-fed pairmates of oral SEB recipients provided additional immunological evidence suggesting that during the 5-hr cross-circulation period, little, if any, fully antigenic SEB or SEB fragment was transferred from the challenged monkey. This observation lends further support to the hypothesis that SEB was not readily or completely absorbed from the gut. SEB antigen evidently does not enter the general circulation during the first 5-hr postadministration period. Some may have been absorbed at a later time in sufficient quantity to initiate an immunological response in the recipient monkey alone. In contrast, a small iv dose of SEB ($5 \mu g/kg$) was capable of producing emesis and eliciting an SEB-antibody response in both members of a cross-circulated monkey pair, demonstrating the effectiveness of a minute quantity of SEB transmitted in the circulation.

Sugiyama and Hayama demonstrated that complete protection from emesis due to oral or iv challenge of SEB was obtained in monkeys through complete deafferentation of the abdominal viscera (7). Sympathectomy alone did not modify the emetic response, while vagotomy alone prevented vomition in animals subjected to oral challenge. These investigators also demonstrated that surgical destruction of the area postrema [chemoreceptor trigger zone (CTZ)] gave a complete refractoriness against oral and iv enterotoxin. However, injection of SEB into the fourth ventricle produced no vomition, suggesting that SEB in the spinal fluid did not act directly on the area postrema as a chemical mediator. Although these findings indicated that the mechanism of oral SEB-induced vomition was based on intact neural emesis reflexes, they offered no explanation as to why vagotomy alone failed to suppress emesis following the iv injection of SEB. The present studies do not define any anatomical

site at which circulating SEB can stimulate an emetic response; they do, however, suggest that initiation of vomition by orally administered SEB is within the gut.

Since emesis can also be induced by iv injection of SEB at a smaller dose, it is possible that SEB administered iv may circulate to neural receptors in the gastrointestinal tract to stimulate vomition. This assumption is supported by the fact that complete abdominal deafferentation abolished emesis in monkeys following either oral or iv challenge of SEB (7). Since radioactively labeled SEB has been shown to accumulate in large quantities in both kidney and liver after its iv administration (11, 12), it is also possible that neural receptors in these organs might be stimulated by circulating SEB. No evidence to support or reject this possibility was obtained in the present study. It is theoretically possible that after oral administration, minute quantities of SEB were absorbed into the portal circulation and removed by hepatic cells. This possibility could lead to neural receptor stimulation within the liver. Based on available evidence, the likelihood of such an event does not seem great; to be compatible with data of the present cross-circulation study, the liver would have to be capable of complete removal of SEB during a single passage of the portal vein blood. Further, Morris et al. demonstrated that there was no significant difference in SEB distribution after peripheral or portal venous routes of administration (12). The data of Morris et al. (12) were obtained with a large single injection of ^{131}I-tagged SEB. To rule out completely the possibility of hepatic accumulation of SEB after oral administration, studies using a more stable SEB tag or highly sensitive analytical techniques for SEB assay will be required.

When induced by other than psychic stimuli, vomition is generally thought to require either a centrally acting humoral stimulation of the CTZ, or a stimulation of unidentified neural receptors in the gastrointestinal tract with impulses traveling along afferent autonomic fibers to the vomiting center. Orally administered SEB appears to initiate a neural reflex arising within the gut that leads to vomiting and intestinal hypermotility. The present study provided additional evidence that vomiting after oral SEB does not involve any centrally acting humoral stimulation.

Summary. Vomition is the most consistent response to oral SEB challenge in the monkey. The technique of cross-circulation clearly differentiates local neural phenomenon from humoral mechanisms. Our results support the theory that SEB-induced vomition follows stimulation of local neural receptors in the gut. The evidence indicates no significant amount of enterotoxin absorption or stimulation of vomition by any centrally acting humoral mechanism.

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